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RELATION OF THE BARBERRY TO STEM RUST IN IOWA

By I. E. Melhus, L. W. Durrell, and R. S. Kirby.

It has been definitely known since 1865 that the European barberry is the alternate host of stem rust (*Puccinia graminis*); yet the exact relation of this shrub to the annual appearance of stem rust of our grains and grasses is not well understood. Tulasne brothers (18) (1847), de Bary (1) (1865) and others early described and figured the morphology of teleutospore germination. They gave little consideration, however, to the questions as to what environmental conditions influence such germination and the production, dissemination, viability and growth of the sporidia,—conditions that have a fundamental bearing on serious attacks of stem rust.

A review of the literature leaves much information still to be desired regarding the manner of infection of the alternate host, the suceptibility of young and old leaves and of the different varieties and species of the genus Berberis, the incubation period, and the influence of climatic factors on aecidiospore production. Of no less importance, locally at least, is the prevalence and distribution of the European barberry and its relation to the development of serious local and general epidemics of stem rust.

It is the purpose of this bulletin to record data and observations on the above factors which have influenced the epidemiology of stem rust in Iowa during the past three years.

SOURCE OF MATERIAL AND METHODS EMPLOYED.

The teleutospore germination studies here recorded were begun by the senior author in the spring of 1910 and continued during 1911 at Madison, Wis. Other work made it impossible for him to take up this problem again until the spring of 1916.

In these studies teleutospores of *Puccinia graminis* on wheat, quack grass (*Agropyron repens*), western wheat grass (*A. Smithii*) and oats and *Puccinia phlei-pratensis* on timothy were used.

Teleutospores of *P. graminis* taken from quack grass and western wheat grass were used most extensively. Plots of quack grass and western wheat grass on the right-of-way of the Chicago and Northwestern railroad heavily infected with stem rust were marked in the fall by means of stakes driven into the

Note: The authors wish to express their appreciation for suggestions and financial support extended by Dr. H. B. Humphrey, in charge of investigations, Bureau of Plant Industry. Acknowledgements are also due to Dr. H. S. Conard, botanist, Grinnell College and Dr. L. H. Pammel, botanist, Iowa Agricultural Experiment Station, for helpful suggestions and assistance in gathering the data recorded in this bulletin.

ground. Teleutospore material was gathered from these plots as needed for the germination tests.

Short sections were cut from ¼ to 1 cm. in length and ½ to 1 mm. in width from the standing straw bearing the spores. These fragments, after being moistened, were placed on glass slides so that a film of water surrounded the spores. When removed from the straw the teleutospores did not germinate well, a fact that was probably first noted by the Tulasne brothers (1847) and later by de Bary (1865). The slide, with fragments of straw, was then held in a petri dish on a piece of moist filter paper until germination took place. In addition to the water cultures, a non-nutrient agar was sometimes used as a coating for the slide to supply the moisture. In still other cases the spore material was suspended over agar in petri dishes.

When large numbers of sporidia were desired for infection experiments, infected straw was moistened and held in a large glass chamber. Within three to four hours large numbers of the spores had germinated and produced sporidia. If allowed to lie in a moist chamber over night at ordinary room temperature those pustules in which the teleutospores had germinated were easily discerned. A glistening white growth formed a blanket over the pustules, as shown in fig. 1. By examination of these with a hand lens or binocular the mass of promycelia could readily be seen, often bearing some sporidia that had not as yet been discharged. Unless these sporidia were gathered within 24 hours, they were of little value for infection experiments.

EXPERIMENTAL DATA ON TELEUTOSPORE GERMINATION.

The teleutospore material already described was subjected to a variety of conditions as to humidity and temperature. Furthermore, the influence of season and state of maturity was considered and also the production and germination of sporidia.

THE INFLUENCE OF HUMIDITY ON TELEUTOSPORE GERMINATION.

The most important factors influencing teleutospore germination are moisture, temperature, and maturity.

First consideration is given to the influence of moisture. It is interesting to note in passing that the Tulasne brothers in 1847 obtained germ tubes from teleutospores placed in water. In 1854, when studing this problem further and using a saturated atmosphere instead of a drop of water, sporidia production was obtained. Blackman (3) (1903) studied teleutospores of *Phragmidium violaceum* and found that, "If the germ

Fig. 1. Straw bearing germinating teleutospores. Note the white masses of promycelia over the teleutospore sori.

tube does not reach the air, it usually grows continuously, the protoplasm with the nucleus being formed at the end of the tube." Later, Magnus (1910) found that teleutospores kept under a thin film of water, form a germ tube instead of promycelium.

Recently Kunkel (12) (1914) has shown that the rust occuring on blackberries, best known as *Caeoma nitens*, responds in this same way. This rust germinates by a long tube when in a drop of water and by a promycelium when resting on the surface of a film of water.

Water is evidently of prime importance to the germination of teleutospores. To determine just how much moisture is necessary tests were made in atmospheres of a known humidity, obtained by using different concentrations of sulphuric acid according to the method described by Stevens (16) (1916).

Two different methods were employed. In the first case the teleutospores on bits of straw were laid on a thin layer of agar placed on a slide. In the second the bits of straw bearing teleutospores were laid on dry clean slides. The agar in the first method served very admirably to hold the spore-laden straw in place. In both cases the bits of straw were moistened, by dipping in distilled water. They were then pressed gently between pieces of blotting paper to remove any extra water, but in each case a thin film was left about the tips of the teleutospores.

Viable teleutospores were placed in different atmospheres where the relative humidity was 58.3, 74.6, 82.3, 95.6, and 100 pct., depending upon the sulphuric acid concentration. The cultures were held from 13 to 21 hours at 20°C. In no case did germination take place where the relative humidity was less than 95.6 pct., unless agar was present. When agar was used, some moisture naturally was available and growth began, but as

the acid in the chamber equalized the moisture content of the agar and the air, the spores were directly influenced. The growth of promycelium began in atmospheres of 95, 82 and 74 pct. humidity but it was soon checked and in no case did sporulation take place. The beginning of growth was doubtless due to the moisture in the agar and the exhaustion of this moisture prevented further development and the promycelium remained as short undeveloped tubes. The most prolific sporidia formation occurred in a saturated atmosphere. At a humidity of 95.6 pct., only a slight production of sporidia was obtained.

It should be noted further that the lengths of the promycelia in the tests made April 27, May 2, and 4, 1917, are greater than those on May 7 as shown in table I. On the former dates the straw was slightly moistened and then dried with filter paper. On the latter date the spores were put dry into the germinating chambers and depended solely on the moisture of the atmosphere for germination. The film of water added in the first case gave an earlier start and accounts for the greater growth.

This suggests an interesting point regarding the relation of water to the type of promycelium produced. In plate I, fig. B, is shown the shape the cells take under normal field conditions. The four basidia and their thread-like stalks average about 80μ in length, the cells are globular or short and cylindrical, and very turgid. When, on the other hand, the spore has germinated in a drop of water or a thin film of water, as on moist agar, the promycelium takes the form of a long, attenuated germ tube. It may reach a length of 500μ. Plate I, figs. A and G, show such promycelia. In certain of the experiments the spores were germinated in a saturated atmosphere, producing the usual short promycelium. After a few hours water was added to the slide and in due time other spores that had not previously germinated put forth promycelia. These, however, were growing in excess water and unlike the ones germinated in saturated atmosphere, took the elongated shape. In these attenuated promycelia, septation of the basidia is irregular; the basidia themselves are indistinct and often less than four in number; the sterigmata are long, sometimes resembling branches of mycelium, and occasionally more than one arises from the basidium. Sporidia production is slight or often wanting.

The promycelia produced in saturated air with no previous wetting showed a different type of promycelium formation. The four cells of the promycelium are globular, turgid and loosely joined, so that on trying to remove them for mounting, they fall apart at their septa on the slightest touch. Promycelia of this type are shown in plate I, figs. C, H and K. It seems clear that sporidia production will only take place in an atmosphere with more than 95.6 pct. relative humidity. Below this point no sporidia are produced. Not only does moisture influence mark-

TABLE I. THE RELATION OF TEMPERATURE TO TELEUTOSPORE GERMINATION

Date	Incubation period	2°C Length of promycelium μ	2°C Amt. of sporulation	5°C Length of promycelium	5°C Amt. of sporulation	10°C Length of promycelium μ	10°C Amt. of sporulation	15°C Length of promycelium μ	15°C Amt. of sporulation	20°C Length of promycelium μ	20°C Amt. of sporulation	25°C Length of promycelium μ	25°C Amt. of sporulation	30°C Length of promycelium μ	30°C Amt. of sporulation	35°C Length of promycelium μ	35°C Amt. of sporulation	Remarks
Apl 27	14 hrs	0μ	0	0	0	320		100		480	*			0		0		Fragments of straw on agar
,,	,,	0μ	0	0μ	0	160		0		0				0		0		,,
,,	,,	0μ	0	0μ	0	0		0		0		0		0		0		Spores on water
,,	,,	0μ	0	*	*	0		0		160		0		0		0		Spores on agar
May 1	,,		0			80	0	480	F	400		240	0	160	0	0	0	
,,	,,		0			320	0	480	F	400	SS	240	0	140	0	0	0	
,,	,,		0			240	0	320	F	320	SS	360	0	160	0	0	0	
May 2	21 hrs		0			480	S	320	S	400	SS	320	F	0	0	0	0	Spores on agar
,,	,,		0			480	S	160	S	480	SS	320	F	0	0	0	0	
,,	,,		0			160	S	80	S	480	SS	320	F	0	0	0	0	
,,	,,		0			240	S	160	S	400	SS							
,,	,,		0			320	S	160	S									
,,	,,		0			320	S											
May 4	19 hrs		0			320	0	480	S	480	SS	160	F	160	0	0	0	Spores on agar
,,	,,		0			160	0	480	S	480	SS	0	0	160	0	0	0	
,,	,,		0			400	0	320	S	480	SS	240	F	160	0	0	0	
,,	,,		0							320	SS							
May 7	,,		0	0	0	80	0	160	S	320	SS	0	0	0	0	0	0	Spores on water
,,	,,		0	0	0	80	0	160	S	400	SS	0	0	0	0	0	0	
,,	,,		0	0	0	80	0	160	S					0	0			
,,	,,		0	0	0	0	0	0	0	48	SS	0	0	0	0	0	0	Spores in moist air
,,	,,		0	0	0	0	0	0	0	48	SS	0	0	0	0	0	0	
,,	,,		0			0	0	0	0	48	SS			0	0			
,,	,,		0			160	S	320	S	48	SS	0	0	0	0	0	0	Spores on agar
,,	,,		0	0	0	160	S	320	S	48	SS	0	0	0	0	0	0	
,,	,,					160	S	160	S	48	SS			0	0			

NOTE—SS—sporulation very prolific.
S—Fair amount of spores produced.
F—Few spores produced.
*—Growth and spore production just noticeable.

edly the extent of germination, but it also modifies, at least to some extent, the morphology of the promycelium and the sporidia.

TEMPERATURE IN RELATION TO TELEUTOSPORE GERMINATION.

Another factor that is closely related to moisture is that of temperature. Many observations and some experimental data are at hand bearing on the influence of temperature on teleutospore germination but relatively little accurate experimental work has been done on the temperature response of the teleutospores of cereal rusts.

Dietel (6) (7) (1912 and 1915) believes that the minimum temperature for germination for *Puccinia graminis* teleutospores is 9.5°C, but states that the number of trials was not sufficient to establish this point. In connection with his studies of *P. larici caprearum*, Dietel notes that the minimum is 6°C. and that at a slightly higher temperature a perfectly normal germination takes place. In *P. graminis* normal germination takes place between the minimum 9.5° and optimum 22°C. but at 23°C. or above only germ tubes are produced, no sporidia being borne. According to Reed and Crabill (15) (1915) the minimum, optimum, and maximum for the germination of the teleutospores of *Gymnosporangium juniperi-virginianae* is 11°, 15° and 29°C., respectively. The upper thermal death point is 30° C. Sporidia production is sparse at 20° with none above 24°C.

To establish the cardinal temperatures for the germination and sporulation of the teleutospores is a more difficult undertaking than in the case of the uredospores. It was found quite impossible to arrange the teleutospores so as to facilitate counting those that did or did not germinate. The promycelia break away readily from the spores if disturbed which again would misrepresent the count made. As described in the chapter dealing with methods, a normal response is not attained if they are detached from the straw. So far, no method has been devised for counting them while attached in the sorus. Length of promycelia is nearly always a moisture reaction, as already described, and as a result it cannot be considered as an exact indicator of temperature reaction. The quantity of sporidia is not an exact indicator because one piece of straw may have more teleutospores than another. Again, each spore has two cells, which may not germinate simultaneously. It is very difficult, therefore, to obtain figures that will represent the influence of temperature as affecting teleutospore germination.

However, in table I are recorded some of the trials made in studying the temperature reaction of teleutospores. It was decided to use the degree of sporidia production as an index of the effect of temperature. This was estimated in each test. The

PLATE I.

A—Characteristic form of promycelium developed in water.
B—Normal form of promycelium and sporidia.
C—Characteristic form of promycelium and sporidia developed in moist air. Cells loosely joined together.
D—Characteristic development of promycelium on moist agar at 15° C.
E—The same at 10° C Nucleii prominent.
F—The same at 30° C Tube twisted and curly.
G—Promycelium produced in water.
H—Promycelium produced in moist air.
I —Promycelium showing tips of sterigmata after sporidia are discharged.
J —Germinating sporidia.
K—Sections of basidia broken off and sending out sterigmata.

data recorded in table I indicate that sporidia production may occur only between 5° and 25°C. and seems most profuse at 20°C. tho the teleutospores will push out a promycelium even at 30°C. The length of the promycelium was greatest at 20°C., which was also the temperature most favorable for sporidia production under the conditions of the experiments.

Not only was the influence of temperature apparent on the production of sporidia, but also on shape, size, and arrangement of the promycelia. The length of the promycelium, however, is very readily influenced by moisture, and therefore probably has only a very indirect bearing on temperature reaction. At 10°C. as shown in plate I, fig. E, the septation is early evident, even in the presence of excess water, and the nuclei are very prominent. Sporidia production seldom results under these conditions, the same characteristics of promycelia being seen 24 hours after the spores are put to germinate.

At 15° as shown in plate I, fig. D, the growth is more normal except that in the presence of excess water the tube-like promycelium is very turgid with a bulb-like end. At 15° to 22°C. the development of the promycelium is as pictured in plate I, figs. A, C, G and H, the nature of the cells depending on the amount of water present. At 25° to 30° germination is slight and no sporidia are produced, the tube-like promycelium tends to be curly and twisted as shown in plate I, fig. F. Results so far obtained, indicate that the teleutospores when mature are not markedly dependent on temperature for their germination. They seem to germinate freely within a wide range. This apparent wide range of the optimum may be due partly to the lack of a means of gauging their optimum reaction to temperature.

The time necessary for teleutospore germination was frequently noted and it was found to be about three hours, altho it often was observed that germ tubes began to show within a period of one hour. As suggested by Dietel, the indications are, that the time necessary for germination increases with the age of the teleutospores, after they have reached their maturity in the spring.

THE SEASONAL RESPONSE OF TELEUTOSPORE GERMINATION

The seasonal response of teleutospores of grain rusts has been known since the early work of de Bary (1) (1865), Plowright (14) (1889) and others.

Dietel found that in central Germany, the teleutospores of *Puccinia graminis* from *Agropyron repens* germinate in the middle of March. When germination begins it takes longer than later in the season. At first it requires 30 hours after the spores are moistened but very shortly germination takes place in 2¾

hours. By June 18 it requires 4½ hours and on July 9 germination is very sparing in 30 hours. By August 14 to 19 no germination takes place, even in 36 hours.

Eriksson (8) (1898a) has shown that the teleutospores of *Puccinia graminis* from *Avena sativa* and *Triticum vulgare* are viable in September, and that teleutospores from *Triticum desertorum* when stored dry for one year and then exposed to natural conditions over winter, germinate normally in 23 hours. Teleutospores from wheat were held for two years in the dry laboratory and then germinated, after being allowed to remain in the open for one winter.

P. graminis teleutospores were formed abundantly in the fall of 1909, in the vicinity of Madison, Wis., on oat stubble, volunteer cereals and quack grass (*Agropyron repens*). Germination tests made the latter part of March gave no positive results. It was not until April 20 that satisfactory germination of teleutospores on quack grass was obtained. Teleutospores taken from volunteer oats, oat stubble and oat straw from a straw stack refused to germinate on this date. Seven days later, April 27, teleutospores of *Puccinia graminis* taken from volunteer oats germinated profusely.

In 1911 an attempt was made to learn how early the teleutospores began to germinate and how long this germination continued. Collections of *Agropyron repens* infected with *Puccinia graminis* were made at various times after February 1 in the vicinity of Madison, and tested as to their germinating capacity. Spores collected during February and March refused to germinate, but on April 14 a collection of teleutospores was made that germinated freely. Some of the trials that were made during April, May, June and July are recorded in table II. It should be noted that after July 13 no germination was obtained altho

TABLE II. THE SEASONAL REACTION OF TELEUTOSPORES OF *Puccinia Graminis*.

Date	No. Cult.	Incubation period Hours	Temp.	Results Germ	No. germ
April 14	2	25	22°C	"	
April 14	4	25	10°C	"	
April 15	7	6	22°C	"	
April 15	7		10°C	"	
April 16	2	3½	10°C	"	
April 20	4	19	10°C	"	
April 21	2	7	10°C	"	
April 22	5	6	10°C	"	
May 10	4	24	20°C		"
May 21	1	2½	10°C	"	
May 21	1	2½	22°C	"	
May 30	1	2½	10°C	"	
May 30	1	2½	22°C	"	
June 10	2	4	10°C	"	
June 20	1		10°C	"	
July 11	2	3	10°C	"	
July 13	2	10	10°C		"
July 13	2	10	27°C		"
July 20	4	24	10°C		"
July 24	4	24	10°C		"

the spores were held in a favorable environment for 24 hours.

It was observed that spores on oat straw lying on the ground refused to germinate. Similar results were obtained in the spring of 1917, when teleutospores of *P. graminis* and *P. coronata* were again taken from the straw of plants that had been harvested and threshed and allowed to lie on the ground in small piles in the cereal nursery. More satisfactory results were secured when teleutospores were taken from oat stubble, altho many collections gave only negative results. The most satisfactory results were obtained when teleutospores were taken from pustules produced on volunteer oats in the fall of the year.

A further study of the failure of teleutospores to germinate in late summer was made in 1917. The first germination was obtained on April 21. After this date, germination was easily obtained from material gathered from the field as needed, until May 20, when the first heavy rain fell, accompanied by rising temperature. On May 26 a second heavy rain fell after which germination was less profuse and only a few spores germinated. An examination of the cells of most of the spores showed that they were empty. Furthermore, on June 27, after a period of considerable more rain, no further germination was obtained and the spores were found to be either immature or empty. This indicated that the spores in the same or different sori do not necessarily germinate all at one time, which confirms Dietel's findings. If their period of viability could be prolonged it would facilitate experimental work very materially.

The influence of low temperature was studied as follows: teleutospores of *P. graminis* from western wheat grass, known to be viable on April 23, were stored at various temperatures.

No. 1. In a cold storage plant at 0°C.
No. 2. In the laboratory refrigerator at 15°C.
No. 3. In the laboratory at 20 to 22°C.
No. 4. In a dry cellar at about 15°C.
No. 5. In a sealed jar buried in the field.
No. 6. Coated with paraffin and stored in the laboratory as in No. 3.

On June 10 cultures were made of material from Nos. 2, 3 and 6; all the material showed germination, but No. 3 germinated very poorly.

On June 26 and 27 cultures were made from all of the lots. Nos. 1, 2 and 4 showed profuse germination and prolific sporidia production. No. 3, on the other hand, germinated very poorly, while Nos. 5 and 6 showed no germination whatever. Trials were made again July 14, but on this date none of the lots would germinate. It would seem that holding the spores at a low temperature prolongs their period of viability to a certain extent.

SPORIDIA PRODUCTION AND GERMINATION.

The production of sporidia follows in three hours after the teleutospores of *Puccinia graminis* are placed under favorable conditions to germinate. As shown in connection with tables I and II, the sporidia are produced most profusely when in contact with a film of water at a temperature of about 20°C.

Prior to sporidia production the basidia are filled with protoplasm which, however, soon segregates to one side of the cell. This movement may be to the same side of all four cells or to different sides, but usually to the same side in normal germination. The wall of the cell at the point where the protoplasm has massed begins to push out, extending gradually until a sterigmata is formed. Under conditions of excess moisture as previously mentioned, the sterigmata may become very long and branched. At the end of the sterigmata an enlargement is formed with a constriction back of it which gradually decreases while the bulbous end increases. The protoplasm of the basidia, in the meantime, migrates into the sterigmata frequently leaving the basidial cell empty and finally concentrating in the bulb-like sporidium at the end of the sterigmata. A final constriction of the narrow part of the sterigmata pinches off the sporidia as described by Buller (5) (1915).

The segregation of the protoplasm to one side of the promycelium and the subsequent pushing out of the cell walls at that side, naturally throws considerable stress upon the cells. The cell wall becomes longer on one side than on the other, resulting in a curved promycelium. Sterigmata bearing the sporidia are borne on the convex side of the curved promycelium, which arrangement effectively points the sporidia away from the straw, so that when they are discharged they are better exposed for distribution.

It would seem that the curving of the promycelium is an adaptation, resulting from difference of pressure and not a positive or negative geotropism or heliotropism, for teleutospores germinated on straw in a vertical position in light or dark produce promycelia curving in all directions, but always so as to point the sterigmata away from the straw.

When fully formed the sporidia are discharged with considerable force as in the Hymenomycetes, so excellently described by Buller (4) (1909). They are frequently thrown a distance of 1000μ. This has been demonstrated hundreds of times by placing pieces of straw bearing teleutospores on media. Under favorable conditions the sporidia will have been formed and thrown away from the teleutospore sorus fully a 1000μ, at which disance from the sorus hundreds of sporidia could be seen in the cultures as a zone surrounding the fragment of straw bearing the germinated teleutospores. In many cases this zone is per-

ceptible to the unaided eye as a white halo. Altho none of the sporidia were actually seen in the process of being discharged, it was repeatedly observed that the sporidia attached to the sterigmata a few minutes later had disappeared. Buller has published a short note as an abstract of a paper he read before the British Association in 1915, in which it is stated that just previous to the discharge of the basidiospore "a drop of liquid exudes where the spore is attached to the sterigmata. The drop varies in size according to the species from one-third to one whole diameter of the spore. Sometimes the drop becomes abnormally large, and then discharge may not take place."

In connection with experiments designed to show how far the sporidia were shot, pieces of straw were laid on a thin film of water and a clean slide placed at various heights above the teleutospore sorus. The sporidia were thrown up against the glass slide one millimeter above the teleutospore sorus. This method of discharge probably helps the sporidia to reach the alternate host by lifting them away from the teleutospore sorus up into the air currents. The sporidia are apparently coated with a substance that causes them to adhere to any smooth surface as, for example, a dry clean slide.

In order to secure abundant sporidia for germination tests, advantage was taken of the fact that the sporidia are forcefully discharged from the sterigmata. Teleutospores were exposed to favorable conditions for germination in a petri dish and a clean dry slide placed so as to receive the sporidia when discharged.

Under favorable conditions of moisture and temperature the sporidia germinate shortly after being discharged. If they are borne on a promycelium in a film of water their discharge is hindered. In this case they may germinate on the sterigmata and produce what might be called a secondary sporidium.

Plate II, figs. G and H, show the type of sporidia germination in water or on the surface of non-nutrient agar. The process of germination of the sporidia is as follows: The protoplasm previously distributed thruout the cell accumulates at one side, and soon the wall pushes out into a lump which resembles a budding yeast cell. The protoplasm aggregates in this germ tube and always appears to be most concentrated at its tip, leaving the sporidia more or less empty as the tube lengthens. The germ tube may arise at any point of the sporidia, such as the side, base, or apex. Likewise, the point of attachment of the sporidium to the sterigmata seems to have no relation to its shape. It may be attached at the side or apex of the ellipsoidal sporidium. After several hours the germ tube grown in water may attain the length of 80μ and is usually curved or twisted. Occasionally the germ tube is branched and often it has an enlargement at the end resembling another spore. Sometimes,

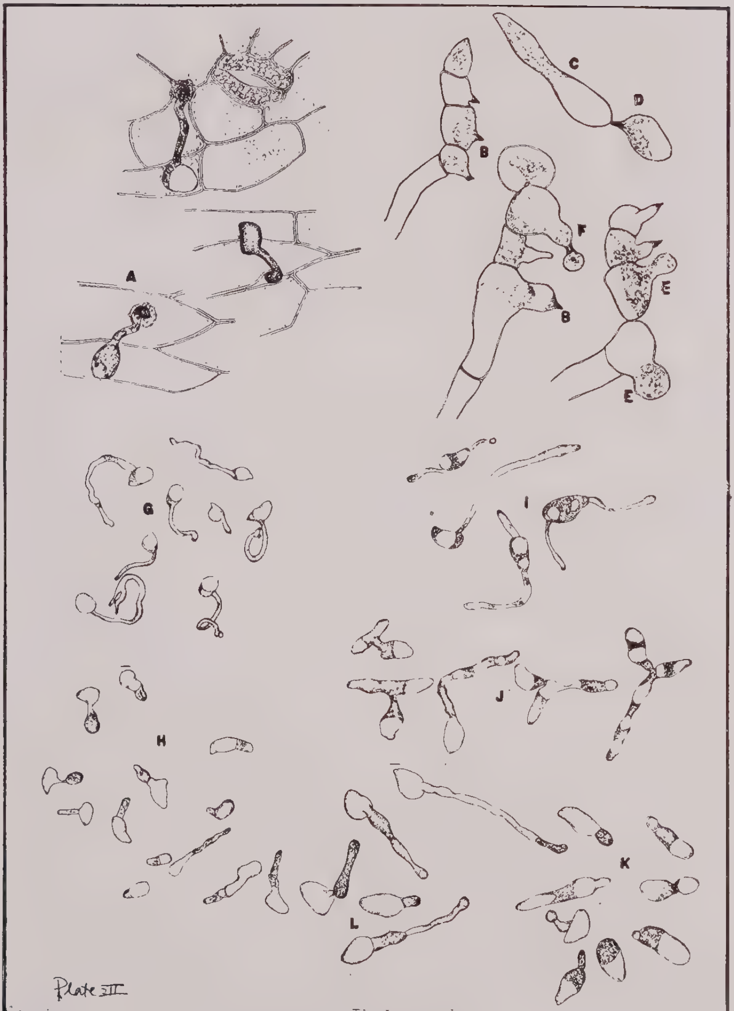

PLATE II.

A—Germ tubes of sporidia penetrating the epidermis of a barberry leaf—both penetrating the cell and forcing between the cells.
B—Hard looking yellow tips of the sterigmata after sporidia are ejected.
C—Sporidia germinating while still attached to basidia.
D—Detached cell of promycelium bearing a sporidia.
E—Sporidia beginning to form.
F—The beginning of the pinching off of the sporidia.
G—Maximum germination attained—in a film of water in 12 to 24 hours.
H—Sporidia starting to germinate.
I —Two days growth of sporidia on beef agar.
J —Two days growth of sporidia on potato agar.
K—Two days growth of sporidia on corn meal agar.
L—Two days growth of sporidia on lima bean agar.

too, a germ tube may be sent out from both ends of a sporidium tho usually one surpasses the other.

Some data have been gathered on the influence of humidity, temperature, and other environmental factors on germination. Experiments to date indicate that drying for a short time prevents the germination of the sporidia. This may well account for the absence in some years of infection on the barberry. Tests were made on June 1, when sporidia were sown on slides on one side of which the spores were only moistened by condensed moisture and on the other side by large drops of water. Under the latter conditions very poor germination and growth were obtained, while in the former germination was profuse. In water the optimum temperature for sporidial germination lay between 15° and 20°C.

By taking advantage of the forcible discharge of the sporidia it was a very easy matter to get the sporidia on media in pure cultures. In a nutrient solution or on a solid medium the sporidia germinated even more profusely than in water, and in many cases were kept in a growing condition for three or four days. The germ tubes grew very long, but in no cases did they show any sign of going down into the media, altho a considerable number of the standard media were tried.

On plate II, figs, I, J and L, are shown tvpes of germinating sporidia on nutrient media. On cornmeal agar the germ tubes were thick and as large as the cell from which they grew. On beef agar the germ tubes tend to be very long and attenuated. Altho it was impossible to get the germ tubes to make a more extensive growth on the media than in water, it would appear from the forms of the germ tube, which are so different from those normally produced in water, that the medium has some effect on their growth.

DATA AND OBSERVATION ON BARBERRY INFECTION

When the relation of moisture and temperature to teleutospore germination and sporidia production had been determined, the question of barberry infection under greenhouse conditions and in the open were considered, also the susceptibility of leaf tissues in different stages of maturity and the time and general distribution of infection on the barberry in Iowa.

INFECTION EXPERIMENTS IN THE LABORATORY.

In connection with the studies of sporidia production and germination, it seemed opportune to devote further study to the infection of the barberry. A survey of the literature revealed the fact that many investigators had secured infection with sporidia, but no one described a method concisely for obtaining abundant infection at will.

For this purpose young barberries (*Berberis vulgaris*) growing in eight inch pots in the greenhouse were employed. Sporidia obtained by the method already described, namely, by placing teleutospore laden straw under favorable conditions for the teleutospores to germinate and catching the sporidia. These were placed in water, and sprayed on the leaves of rapidly growing barberry bushes. For the next 48 hrs. the plants were held in a saturated atmosphere in a moist chamber, 4′ square and 1½′ high. During the remaining portion of the infection period the plants were held on a bench in the greenhouse where the temperature was about 15°C. at night, and from 22° to 25°C. during the day, with the humidity as a rule between 40 and 50 pct. during the day and between 60 and 70 pct. at night.

Fig. 2. Shoot of European barberry, (Berberis vulgaris) showing aecidial infection produced by spraying the plant with a water suspension of the sporidia. Heavy infection may be readily obtained in this way.

Signs of aecidia began to appear after eight days and aecidia with mature spores were at hand after 12 days. Thus far in the infection experiments only the young leaves, at the time the plants were exposed, became infected and produced the aecidial stage. Many of the leaves had from 4 to 10 sori. The amount of infection obtained is shown in fig. 2.

When the leaves of *Berberis vulgaris* have taken on a dark green color and crisp, hard appearance, infection apparently does not take place. Repeated attempts in the greenhouse and field under

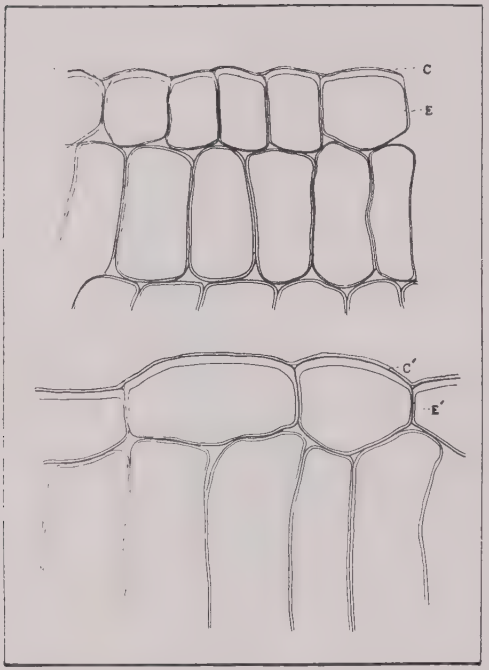

Fig. 3. Sections of barberry leaves showing the difference in thickness of epidermis and cuticle. C, cuticle on young leaf; E, epidermis on young leaf; C', cuticle on old leaf; E', epidermis on old leaf.

varied moisture and temperature conditions failed to produce infection on the more mature leaves.

The two surfaces of young barberry leaves are equally susceptible altho the stomata are only on the lower surface. The germ tubes of sporidia, however, are not dependent upon the stomata as an avenue for entering the leaf. They readily force their way between the cells of the epidermis down into the leaf, altho some germ tubes were even found penetrating the cell walls of the epidermis as shown in plate II, fig. A. Before penetrating the leaf the germ tube enlarges at the end and the protoplasmic contents become massed at that point. Such enlargements may occasionally be found on germ tubes produced in water cultures as well as on the host leaf.

The marked difference in the susceptibility of young and old leaves of the barberry may be due to the thickness of cuticle and epidermis, considering that infection is accomplished by penetration of the tissues and not thru invasion of stomata. In fig. 3 sections of old and young barberry leaves are shown, illustrating the difference between the old and young leaves as to thickness of cuticle and outer cell wall. Therefore, the age of the barberry leaves at the time of sporidia production may account for the variation during certain saesons in the amount of the aecidia on the barberry.

In fig. 4 shoots of European barberry are shown with leaves just unfolding. It is in this stage and shortly afterward that

the tissues seem to be the most readily penetrated by the germinated sporidia. It is at this time too that the teleutospores first begin to germinate profusely outdoors.

INFECTION IN THE OPEN.

Natural infection out of doors follows very soon after the teleutospores begin to germinate freely in the spring. In 1917 the teleutospores of *Puccinia graminis* first germinated freely on April 21 and the first infection on the barberry was found on May 5. This seems to be the prevailing condition each season as shown in the following table setting forth observations made at Ames, Iowa, during 1916, 1917, and 1918.

Spore stages	Host	1916	1917	1918
Germinated teleutospores	Stubble and grasses		April 21	May 3
Aecidium	Berberis vulgaris	May 7	May 5	May 8
Uredospore	Oats	May 21		
" "	Wheat		June 15	June 14
" "	Agrostis alba			May 20
" "	Hordeum jubatum			May 25
Teleutospore	Agropyron repens			June 21

Altho the data in this table are rather limited they indicate that usually from 5 to 14 days elapse between the first date at which the teleutospores germinate freely and the first appearance of the aecidium. It also seems that the aecidia make their appearance before the uredospore stage.

A similar correlation between the advent of the aecidia on the barberry and the uredospore stage of *Puccinia graminis* has been recorded at St. Paul, Minn., by Freeman and Johnson (10) (1911), 1907 to 1909 inclusive.

	1907	1908	1908
Aecidium	July 15	June 1	June 14 to 26
Uredospore	July 26	July 3	July 5

Survey of 1917: In 1917 the first aecidia were found on the barberry on May 5. After this date it was readily possible to find aecidia until July 15. Altho every county in the state was not visited, aecidia were found on barberry bushes in 23 different counties. No particular section of the state seemed to be especially free from infection. About 20,000 European barberry bushes were found in 35 counties in all quarters of the state and the aecidial stage was collected in 23 of these counties. Altho the survey in 1917 was not sufficiently extensive to reach every county, sufficient data were gathered to show that the distribution of the barberry was general and that they were commonly infected with the aecidial stage of stem rust.

Survey in 1918: In 1918 the first aecidia were found on May 8 at Burlington, May 12 at Fayette, May 14 at Jessup. This was 3 days later than the earliest collection in 1917. By May 20 the aecidia were found to be opening at Clinton, Blockton,

Fig. 4. Shoots of European barberry showing leaves emerging from buds. It is at this stage and shortly after that the tissues are softest and infection by sporidia takes place.

Donnelson, Charles City and Mt. Pleasant and on May 25 aecidia were found quite fully formed at Wyoming, Dubuque, Maquoketa, Mason City and Fairfield. The uredospore stage of *Puccinia graminis* was first found on May 25 on *Hordeum jubatum* near some infected barberries.

In every case the aecidia found showed every sign of being the initial stage. Within a period of 17 days after the first infection was found, aecidia developed on barberry bushes thruout the eastern half of the state. Unfortunately no survey was made of the western half at this time so no statement can be made as to the condition that prevailed there. However, it is very significant that within a period of 17 days or less, aecidia should be produced generally over such an extensive area. Certainly the uredospore stage had ample opportunity to begin its development at many points.

Young aecidia were found in abundance until July 10 but after this date they were rare. At McGregor on July 26 in a cool shaded place, a single young aecidium was found on a bush that showed no other signs of infection. This was the last sign of young aecidia until August 14 when 16 young aecidia were found on a hedge belonging to G. Ramsey at Grinnell. Many dried up aecidia were present on this hedge, but no young aecidia, except on three bushes at one end where they were present in all stages of development.

The complete reports from counties where infection was lo-

cated would indicate that in the majority of cases, aecidiospores were discharged in May and their dispersal kept up until about July 10.

Fig. 5 shows 99 stations at which aecidia were found on barberry bushes. The survey started March 15, with the result that the southwestern and several of the central and eastern counties had been surveyed before it was time for the aecidia to appear on the bushes. After aecidia were first found on the barberry, the scouts found the aecidium in 94 pct. of the counties visited. In the four following counties, Hamilton, Hardin, Marshall and Monroe, no infection was located, but in these cases the survey was not completed until late in August and September.

Prevalences: In connection with the survey for the distribution of the aecidium on the barberry it was observed that there was a wide variation in the percentage that were infected at any one time. The greatest amount of infection on the barberry was found on rather old hedges growing in the country near Mason City, LeClaire, Clinton, Dubuque and Allison, where the bushes were surrounded by grasses or grains abundantly infested with teleutospores. In such cases it was not uncommon to find from three to nine aecidia on a single leaf and the number on a single bush would often be more than one hundred.

The next five cases, in degree of infection, occurred at Waterloo, Knoxville, Chariton, Grinnell and Osceola. Here the number of aecidia on a bush varied from 50 to 100. In these cases

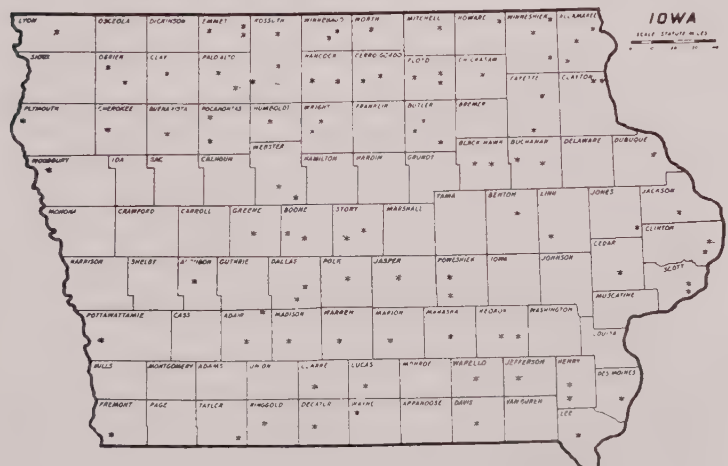

Fig. 5. In this figure are shown the points at which the aecidial stage was found on the barberry in Iowa during the season of 1918. The asterisk indicates where infections were found.

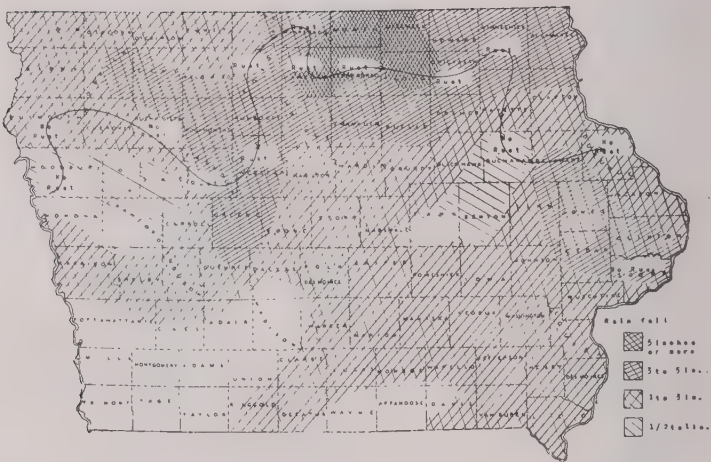

Fig. 6. Map showing the relation of rainfall to the presence of the aecidial stage of stem rust in northern Iowa, August, 1917. The aecidia were most common in sections having the greatest rainfall.

the barberry bushes occured not in the country, but on lawns in the outskirts of the cities cited. The importance of having teleutospore infested grasses near the bushes was well demonstrated in Auld Park at Knoxville on June 21 where 12 bushes were scattered over an area of two city blocks. The park was located on the north edge of town and considerable shrubbery was present. In the west half of the park a barberry bush was located, at whose base a number of culms of *Agropyron repens* were found, covered with teleutospores. As far as could be determined there were no teleutospore infested grasses near the other bushes. This bush had 90 infected leaves. A barberry bush 10 ft. to the east had 8 infected leaves. Two other bushes growing 100 ft. northwest and 200 ft. east had three and one infected leaves respectively. No infection was found on any of the other eight bushes.

Another case is the one figured in fig. 12 and located east of Mason City. The *Hordeum jubatum* and *Agrostis alba* growing under and beside the hedge along the road was very generally infected with teleutospores. On June 27 most of the barberry bushes along the road had over 100 infected leaves and some single leaves were covered with as many as nine aecidia. The infection on the bushes between the woods and corn where infected grasses were absent decreased rapidly as the distance from the road increased. On the north side of the woods about 30 rods from the road only three or four aecidia could be found on a few of the bushes.

In most of the larger towns, nearly all of the infected barberry bushes were near the edge of town. This was the case in Des Moines, Sioux City, Cedar Rapids, Davenport and Waterloo. However, in Dubuque and Burlington over 12 pct. of the plantings examined, were infected, without regard to their location in the city.

In such smaller towns as Parkersburg, Forest City, Emmetsburg, Algona, McGregor and Mt. Ayr, from 20 to 100 percent of the barberry plantings were infected.

The fact that towns are full of natural obstructions, such as houses, bushes and trees, and also that blue grass and pavement have replaced the grass hosts and cereals on which the teleutospores are produced, no doubt explains why the number of infected barberry bushes found in the cities is less than on those growing in the towns and country.

Relation to moisture: One of the controlling factors in the prevalence of the aecidial stage seems to be that of moisture. It was noted on several occasions that soon after a rain a crop of aecidia developed. This is quite clearly illustrated in fig. 6, which traces a trip made by S. M. Dietz across northern Iowa from Sioux City to Dubuque in August, 1917. At this time he found no aecidia thruout Woodbury county altho at different points over 300 bushes were examined. The rainfall in this section was very light. Fig. 6 shows that the rainfall for July in Woodbury county was only between 0.5 and 1 inch. The rainfall for May ranged from 2 to 6 inches and aecidia were prevalent in June.

Fig. 7. Seedlings of European barberry (Berberis vulgaris) found growing under a large bush in August, 1917. It is interesting that these seedlings were heavily rusted.

In the zone where the rainfall ranged from 3 to 5 inches the aecidia were common while in the area (Cerro Gordo and Floyd counties) with more than 5 inches rainfall the aecidia were very common. Upon leaving this zone the amount of infection decreased with the decrease in rainfall. It would seem that rainfall is an important limiting factor in the production of the aecidia of stem rust (*Puccinia graminis.*)

As already mentioned, the first barberry infection was found at Ames, Iowa, May 5, 1917. Aecidia continued to develop until August 6. After the middle of July, however, they were not very numerous and it was only by searching carefully that aecidia could be found, and these always on the barberry leaves nearest to the grass growing about the bushes. The seedling barberries seemed to afford very favorable material for infection and when present they were nearly always infested. Fig. 7 shows four seedlings collected July 15 that were infected. The aecidia found after July 15 were much smaller than those found earlier and often only a few cups would be developed. It seemed that as the season advanced and the temperature went higher the aecidia became smaller. In the greenhouse and out of doors in the month of June, when there was abundant moisture and the weather was rather cool, large aecidia with exceedingly well developed peridial cells formed, while during the last half of July and in August the aecidia were very much smaller often consisting of only one to four cups.

EUROPEAN OR COMMON BARBERRIES.

PREVALENCE AND DISTRIBUTION.

During the past two years an effort has been made to locate all the barberry bushes in Iowa. At the present writing this task is far from complete altho over 300 cities and towns in the state have been surveyed and 132,673 bushes have been found growing on private and public grounds according to the 1918 records. In 1917, on a preliminary survey, about 20,000 were located that are not shown in fig. 8, which shows the number of barberry bushes located in each county in 1918. It should be stated that in most cases the survey is not complete and that the figures given are doubtless in many cases inaccurate from the standpoint of the actual number of bushes in the county. Barberry bushes are continually being reported in the small towns and villages as well as in the country. There is a good reason to believe that this number will be doubled and possibly tripled as the survey continues.

It is interesting to note that over one-half of the bushes were found in the two eastern tiers of counties along the Mississippi river. This may be accounted for in part by the fact that this part of the state was first settled and also more survey work

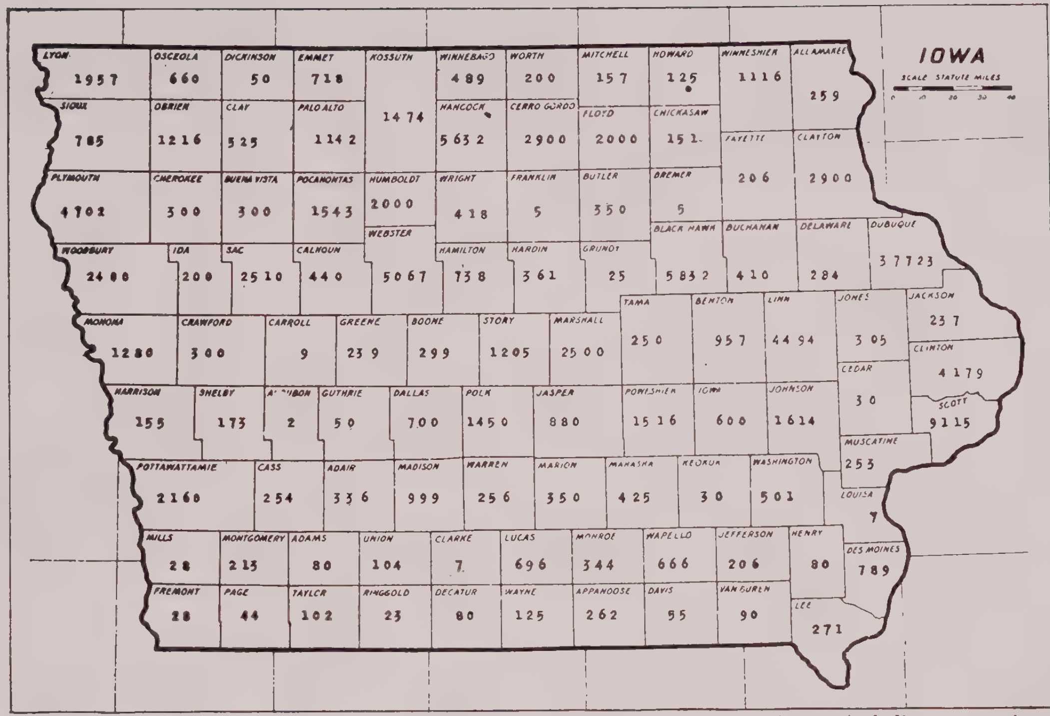

Fig. 8. Distribution of European barberries located in Iowa, survey of 1918, not including nurseries.

has been done there. Outside of a few of the larger towns, barberry bushes were found to be less abundant in the southern than in the northern part of the state.

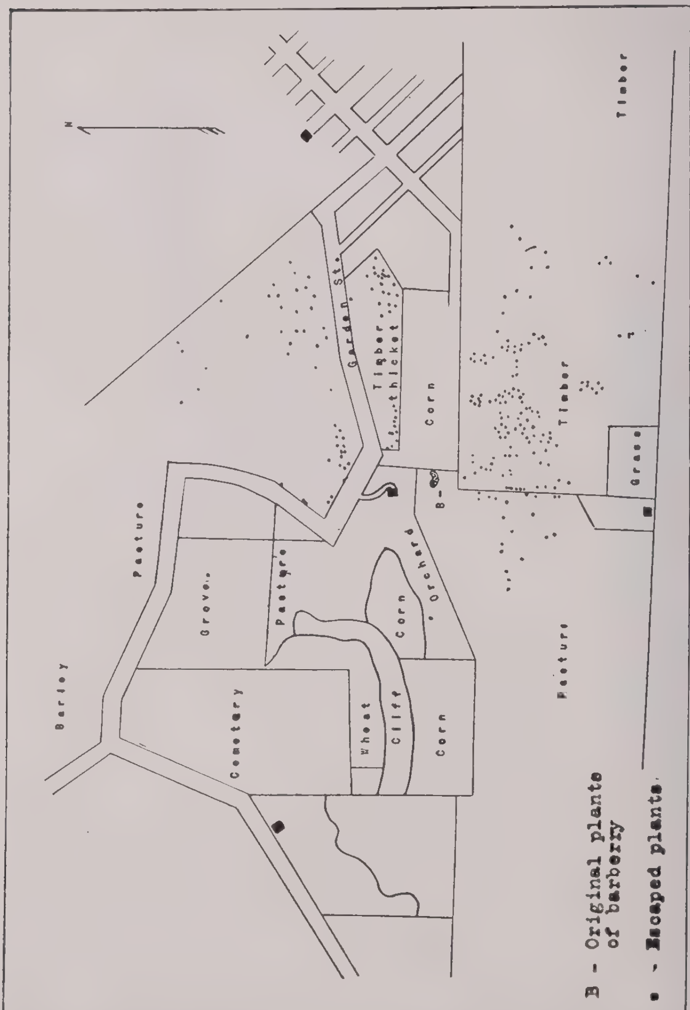

Fig. 9. This illustration shows the distribution of the shrub as determined by the surveys carried on during 1918, (not including nurseries.) It is obvious that the largest number of bushes were planted in the larger cities. The counties along the Mississippi river had more barberry than any other section.

This consideration of the number of bushes found in Iowa does not take into account the bushes that were dug up and destroyed by the owners due to the influence of the press. Neither does it take into consideration the thousands of bushes, involving in some cases acres of nursery stock, that were destroyed by the nursery men. Altho Iowa has approximately 229 nurseries, only 10 of these now have barberries growing in them.

ESCAPED BUSHES.

Barberry bushes occur not only as plantings but also as escaped plants about old hedges or single plants. The most striking case that has come to our attention is at McGregor. The distribution of escaped and original plants is shown in Fig. 9. The planting fro which the other bushes originated stood in the outskirts of the city. The seeds from these old plants were doubtless carried by the birds and dropped in the rough, sparsely wooded timberland in the immediate vicinity. Most of the escaped bushes were found below over-hanging branches which served as resting places for the birds. Altho the surrounding country has been surveyed several times and over 500 escaped bushes have been located, no bushes have been found more than a mile from the original planting.

Near Mason City, escaped barberries were found in a planted grove, surrounded on two sides by a large double rowed barberry hedge. Here also the birds apparently distributed the seed. One bush was found about 300 feet from the hedge along a fence growing in the bluegrass sod.

Conditions were much the same at Clinton where barberry bushes have escaped from a hedge said to have been planted 40 years ago on the place owned by Mrs. E. E. Pearce. Over 400 bushes were found scattered in the woods and pastures for a distance of three-quarters of a mile from the original plantings. The bushes were of all sizes, ranging from one-year-old seedlings to old bushes 10 ft. high. These bushes were mostly located along the fences and under the trees, again suggesting that the seeds were scattered by the birds.

A similar condition existed seven miles northwest of LeClaire where a considerable number of barberry bushes were found in a piece of native timber at a distance of one mile from the original hedge. Escaped bushes were also found in an old orchard near the hedge. In a like manner barberry bushes have been scattered about original plantings at Vinton, Searsboro, Jefferson, Indianola, Cascade and Montpelier. Fig. 10 shows a picture of the original plantings of barberry on the F. D. Lowry place near Montpelier. This old hedge is 12 ft. high and 16 ft. wide. Fig. 11 shows one of several escaped bushes out in a pasture about 30 rods from the original planting under a large tree.

A case at Garner merits special mention. In this instance the barberries were left in a nursery that was abandoned. Altho oiginally the bushes stood in nursery rows, today they occur promiscuously thruout the remainder of the nursery, which has grown up into a young grove of maples and shrubbery. A small orchard and surrounding grove of box elder and willow across the road is also well infested with escaped barberries, the majority of which are to be found in the woodlot adjoining the orchard.

It is perfectly clear that the barberry has escaped from cultivation in Iowa at many points. In every case, however, the original plants serving as centers are of long standing. The birds seemed to be quite effective agents for the distribution of the seed. Escaped plants have been found in native timber, planted groves, along fences, in old poorly kept orchards, and in abandoned nurseries, but never more than a mile from the original planting. No case has been found where barberries have established themselves in cultivated land.

THE SPREAD OF STEM RUST FROM THE BARBERRY TO GRASSES AND GRAINS.

During the past summer evidence has been collected which suggests quite clearly that the European barberry bushes may start local epidemics on the grasses and grains in their immediate vicinity in much the same way as described in the early

Fig. 10. Old hedge of barberry on the farm of F. D. Lowry, at Montpelier, Iowa. Photographed by J. H. Parrmann.

Fig. 11. Escaped barberry under a tree in a pasture on F. D. Lowry's place at Montpelier, Iowa. Photographed by J. H. Parrmann.

European records. Sixteen such local epidemics or infection centers were found during June, July and August of 1918. Many of these are quite similar in their development so only eight cases will be described in the following pages. Doubtless there were others that did not come to our attention.

Such a local epidemic is well illustrated at Mason City about a hedge on the Phoebe Brentner farm. The barberry bushes are planted in two rows about three feet apart along the road and around a farm grove surrounding the farm buildings. This hedge is now about six feet tall and ten feet in width. Grasses grow in among the bushes and along the roadside. The adjoining land is all under cultivation or used for pasture. The field immediately adjoining the hedge was either planted to corn and beans or in pasture this past season.

The aecidial stage was very abundant on this hedge in May and June. The grasses immediately surrounding the hedge were heavily infected with teleutospores of stem rust produced during 1917. The aecidial stage developed in abundance during May and June.

On June 28 a detailed survey was made of the rust situation about this hedge on the grasses and grain, the findings are shown graphically in fig. 12. On this date, the *Hordeum jubatum* and *Agrostis alba* near the barberry bushes along the road, were so heavily covered with teleutospores that the black color was

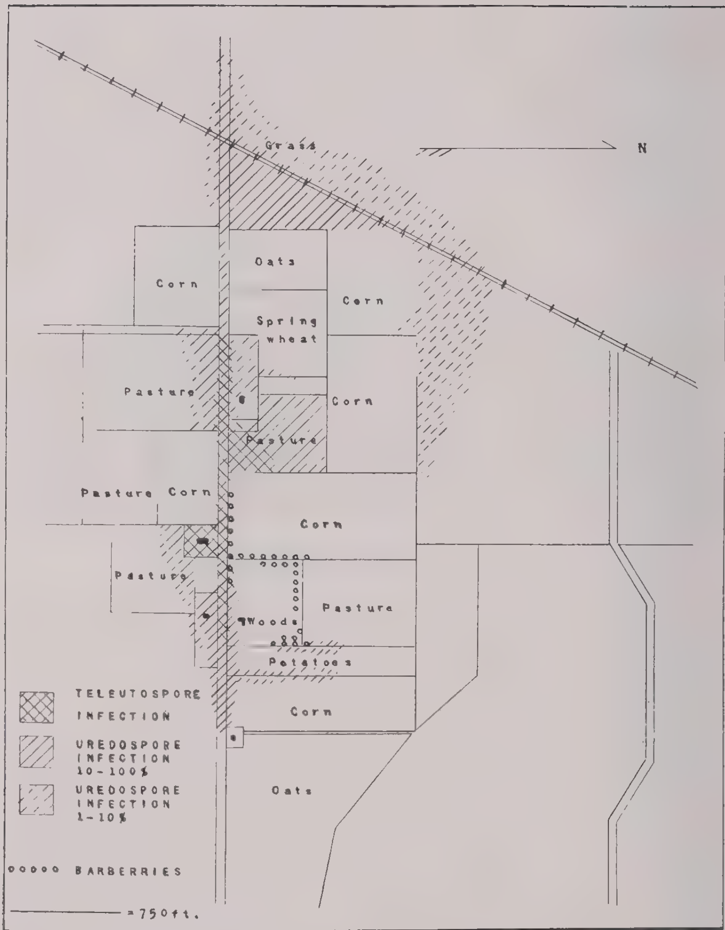

Fig. 12. An old barberry hedge on the Phoebe Brentner farm at Mason City, about which a rust epidemic was well advanced on June 28, 1918. The rust spread in a northwesterly direction from the hedge on the wild grasses.

readily discernible while driving by in an automobile. No teleutospores were found on *A. alba* at a distance of over 100 ft. from the hedge, but on *Hordeum jubatum* teleutospore sori decreased in amount as the survey extended east or west along the road and finally disappeared at a distance of 1200 ft. west of the hedge and 200 ft. east. Beyond this zone of grasses infested

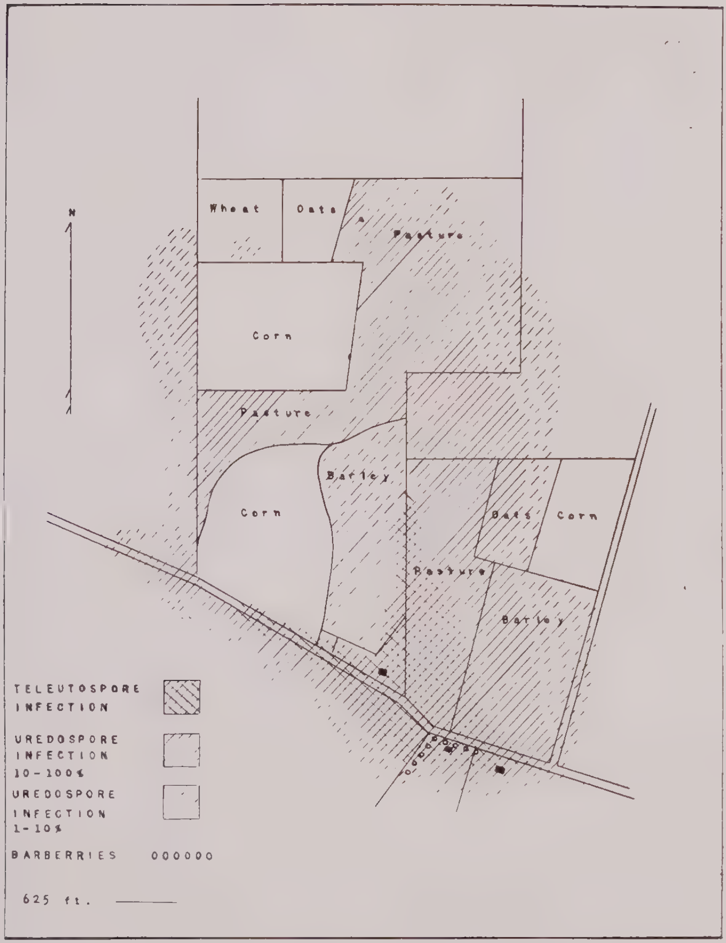

Fig. 13. The rust spread from the John Boldt hedge at Dubuque to barley and wild grasses in a northerly direction from the hedge. It followed the grasses along the roadside and in the pasture east of the hedge. A wheat field near the barberry bushes remained quite free from rust.

with teleutospores, was a bordering area having grasses infested with uredospores.

Hordeum jubatum was the most common grass in this area, and uredospore sori were found in decreasing amounts to a distance of 2900 ft. west and northwest of the barberry hedge. Beyond this area the grass was free from infection. To the east,

the area of infested grasses extended down the road a distance of 900 ft. North of the woods only a mere trace was found on this grass north and east of the bushes. On *Elymus robustus* and *E. striatus* uredospore sori were only traceable for a few feet from the hedge.

A field of spring wheat just beginning to flower was located 900 ft. north and west of the barberry hedge. Only a slight amount of the uredospore stage was found on the eastern edge of this field, at the time this survey was made. The subsequent rust development in the wheat was slow, due to the weather being unfavorable for the development of stem rust. Sixteen hundred feet west and 700 ft. east, oats showed no signs of rust. The grasses and grains beyond the infected area were examined, but only a trace of rust could be found.

The rust situation surrounding this hedge shows clearly that infection took place earlier on the grasses immediately surrounding the bushes. Teleutospore production follows uredospore formation. The abundant aecidia on the barberries doubtless were responsible for the initial infection, which spread in every direction from the hedge where susceptible host material was at hand. There was a marked gradation in the degree of infection as the distance from the barberries increased. The rust seemed to spread northwest from the hedge much faster than in any other direction.

Another case where the barberry served to start a local epidemic of stem rust on grasses and barley was about the John Boldt hedge near Dubuque. A detailed survey of the rust situation about this hedge was made on July 14. Aecidia were first found on May 24 and they continued to develop until the hedge was eradicated several weeks later. As shown in fig. 13, the hedge grew near the road only a short distance from two fields planted to barley.

On July 14 the stems of *Hordeum jubatum, Agrostis alba, Agropyron repens* and *A. tenerum* growing near the barberry bushes were badly discolored due to the profuse teleutospore production. The barley straw across the road from the hedge was quite black, for 250 ft. out into the field, due to the profuse development of teleutospores. Beyond this area the number of uredospore sori decreased until only traces existed at 900 ft. east, and northeast of the hedge. Uredospore sori occurred on the grasses for a distance of 4600 ft. north and northwest, in the case of *Agropyron repens* and *Hordeum jubatum* and for 3500 ft. on *Agrostis alba.*

In the barley field 100 ft. north of the hedge, there was about five percent of rust, except in an area of low ground where it had increased to about 30 percent. Volunteer rye in the spring wheat field 4000 ft. north, was heavily rusted while only one uredospore sorus was found on the wheat. The area of uredo-

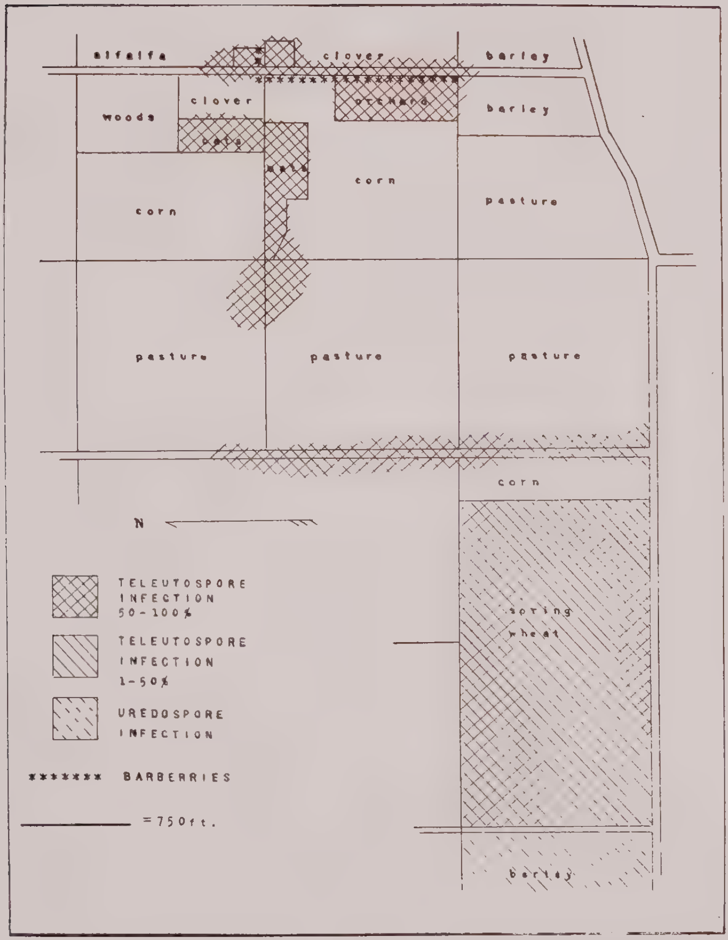

Fig. 14. About the barberry hedge on the property of J. H. Johnson at LeClaire, the grasses and oats and wheat rusted badly. The limits of the epidemic were marked and very distinctly set off from the surrounding region.

spore infection surrounds the zone of teleutospore infested grasses and grains in exactly the same way as shown in fig. 12. Beyond the area of uredospore infection, which was a marked zone, only a trace of stem rust could be found. The marked absence of stem rust on the wheat, and its abundance on rye and quack grass suggests that *Puccinia graminis tritici* was absent and that only *P. graminis agrostis* and *P. graminis secalis* were

present. It should be stated also, that it was experimentally determined, that the *P. graminis secalis* was the dominating form in this locality.

Near Le Claire a barberry hedge started a local epidemic that seriously injured a field of oats. This hedge was about 80 rods long and one of the oldest plantings in Scott county. A diagram of the hedge is shown in fig. 14. It was eradicated by the owner J. H. Johnson during November and December at considerable cost. The location of the hedge and its relation to surrounding grain fields is shown in fig. 14. Seeds from this hedge have been dropped by birds in a nearby orchard and in a woodlot nearly a mile from the hedge. The size and density of the hedge was similar to fig. 10. The grasses and grains about this hedge were examined for the presence of rust on two different occasions, July 23 and August 7. On the last named date traces of stem rust were common on grain and grasses all over Scott county, so that the demarkations between areas showing different degrees of rust were not striking. When the survey was made on July 23, *Hordeum jubatum* was badly infested with the teleutospore stage not only about the hedge but also along the road sides fully 160 rods away. All the *Agrostis alba* within a radius of 80 rods of the hedge was equally infected.

A field of oats 400 feet west of the hedge showed 65 pct. of stem rust. The oats from this field gave a low yield. Another field of late oats to the north, that was still green on July 23, showed 90 percent of stem rust and the crop was very seriously injured. It was so poor that it was not considered worth threshing. The wheat three-fourths of a mile west showed 30 pct. stem rust and a barley field one mile east showed three percent stem rust of the uredospore stage.

The two fields of barley south and southeast of the hedge escaped serious infection. It is believed that this was due to one or more of the following circumstances. First, that the biologic form going to barley did not make as rapid headway in the early part of the season, second, that the prevailing winds prevented these two barley fields from being exposed to infection or third that the barley matured before the rust gained sufficient momentum to attack the grain. The barley matured early and was harvested July 15. It is interesting to compare the amount of rust on these two fields with that on the field west of the wheat field, nearly a mile from the barberry hedge. This field of barley was later than the other two. It was green on July 23. The rust became quite prevalent in this field, yet there was insufficient time for serious epidemic to result.

The epidemic induced by this hedge has much in common with those figured in figs. 12 and 13. It differs in the respect, however, that the biologic form on oats became very prevalent and caused serious damage to oats near by. It is also clear that the

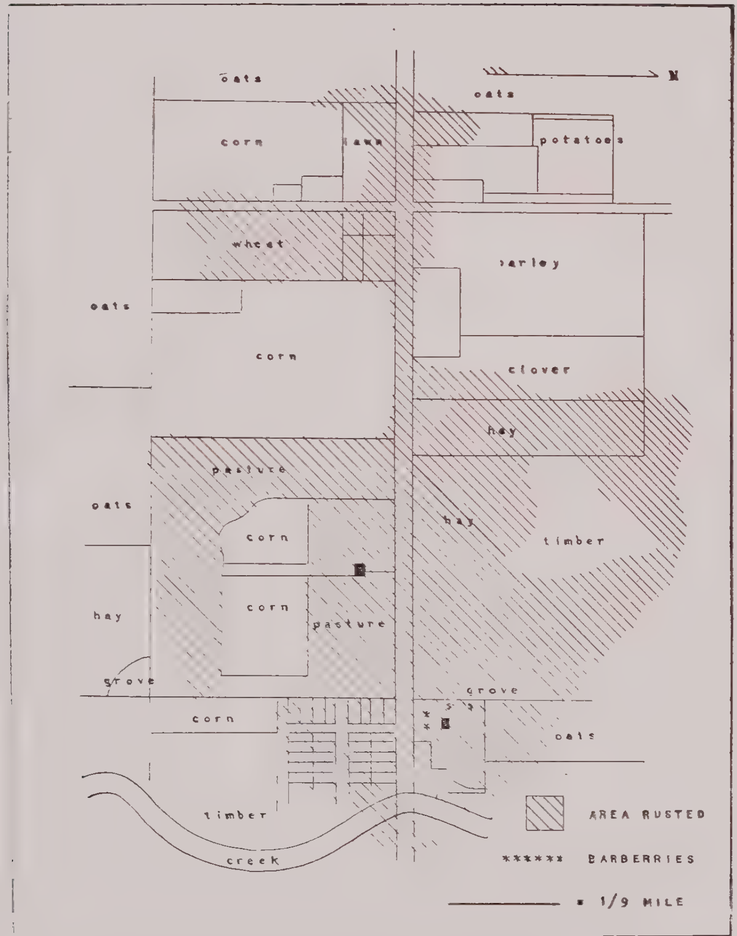

Fig. 15. Map showing distribution of rust from barberry to wild grasses and grain at Riceville, Iowa. The barberry bushes on the edge of Riceville are shown surrounded by a rust epidemic, which followed the grasses over to the oats and wheat fields.

area of rust infection spread further from the barberry hedge in a westerly direction.

In the three examples, cited to show the relation of stem rust to the barberry, the bushes occured as old hedges in the country. The question naturally comes up as to what role barberries in the outskirts of towns and cities may play in aiding the spread

of stem rust. Such a case was found on the outskirts at Riceville on the M. Thieleus place. Two clumps of purple leaf barberries were growing on a very well kept front lawn. The bushes were about five feet high and stood within 75 feet of the street where *Hordeum jubatum, Agropyron repens* and *Agrostis alba* occurred in great abundance. On July 13, when the senior author visited these bushes they were abundantly infested with the aecidial stage of stem rust. The grasses in the street were heavily infested with the uredospore stage. At this time it was not readily possible to find stem rust on either the grasses named above or the grains but here near the barberry bushes it was very abundant. It was possible to trace the infection along the street and road as well as across the street into some vacant lots and pasture for 450 feet where squirrel tail grass was quite common. The oats north of the house were not examined. The two oat fields west of the corn and potatoes were free from rust, but the wheat on the other hand showed about 5 pct. stem rust.

On September 2, H. S. Conard made another survey and found the conditions as shown in fig. 15. The barberries seemed to be the point from which the local epidemic started. It spread westward along the road on *Hordeum jubatum, Agropyron repens* and *Agrostis alba*. The oat field near the barberries became infested but the two south and west remained quite free from infection. The wheat became generally infected while the barley escaped, due to its early maturity. Be this as it may, it is clearly shown that barberries occurring on lawns in the outskirts of cities may serve to start an epidemic of stem rust. The damage that the same may do will be limited only by the weather conditions.

The rust developments at Riceville were not exceptional, a similar local epidemic starting in the city of Knoxville, about barberry bushes growing on a lawn at the edge of the city. The grasses along the railroad track afforded opportunity for the barberries to become well infected. The rust spread into the park and eastward along the railroad as shown in fig. 16. Here as in the case at Riceville a wheat field about 100 rods away from the hedge became badly rusted. Due to intervening fields, it was not possible to trace the infection on the grasses all the way from the barberries to the wheat field. Nevertheless it is not impossible that spores were carried to the wheat from the local epidemic about the hedge. The epidemic was spreading in an easterly direction. Possibly the trains aided the spread of the uredospores.

Still another example of barberries in the edge of a city serving to start a rust epidemic is illustrated by the condition at Forest City. In this case, 50 purple-leaved barberries in a hedge were growing on the lawn of Andred Thursen in the northwest part of the city. Aecidia occurred in abundance on the hedge

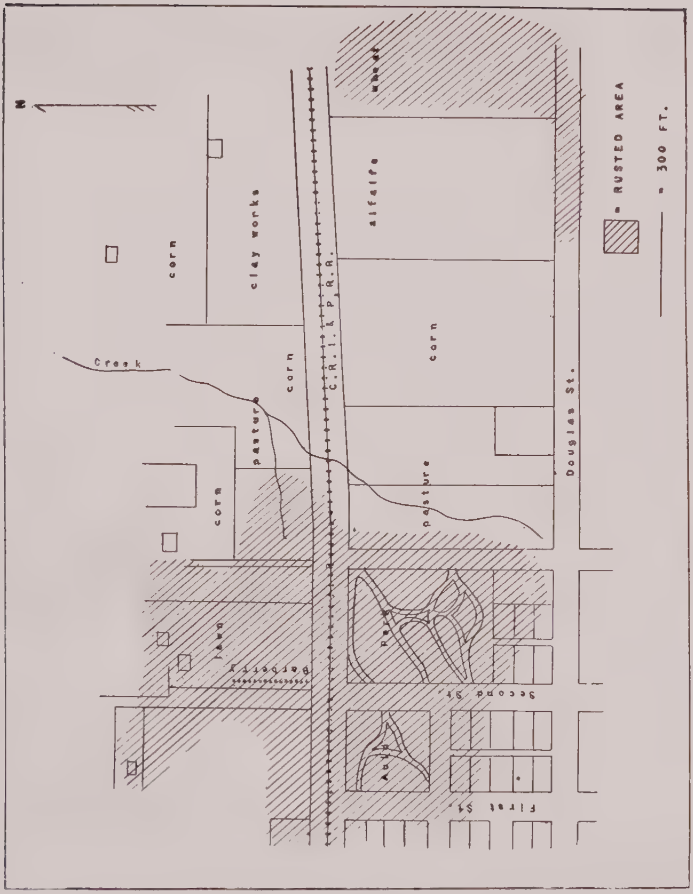

Fig. 16. Map showing a local epidemic that developed in the outskirts of Knoxville. The rust spread from the barberry to the wild grasses. A wheat field east of this center became badly infested with stem rust.

until July 30. *Hordeum jubatum, Agropyron repens* and *Agrostis alba* near the hedge were heavily infected with the teleutospore stage of stem rust. Outside of the shaded area in fig. 17 there was little stem rust, very much less than within the shaded area. In this case as in the one at Riceville, a local epidemic developed that was very distinct and readily outlined.

A somewhat comparable case as to size of the infected area was found in the outskirts of Grinnell, fig. 18. A hedge of 200 plants on the Ramsey place served as a center. The culms of

the 1917 crop of grasses about the bushes were heavily infected with teleutospores and abundant aecidia developed on the barberries in July. Old aecidia were very much in evidence on August 13 and the 1918 crop of *Hordeum jubatum*, *Agrostis*

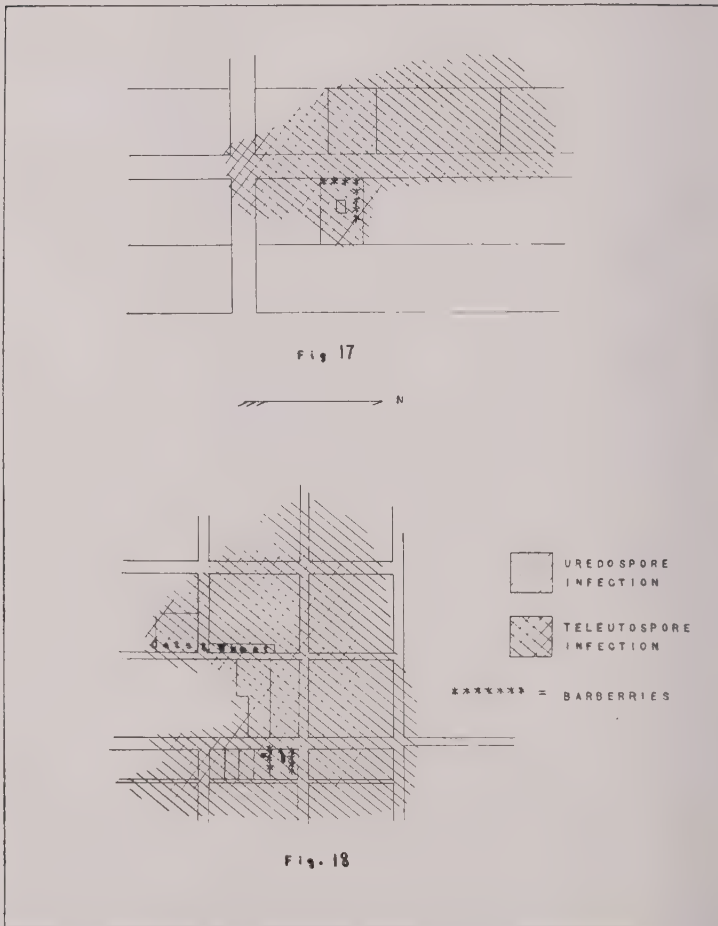

Fig. 17. The epidemic in this case started from barberry bushes growing on a lawn in the edge of Forest city. This epidemic did not trace far into
Fig. 18. Here the stem rust was very bad about barberry bushes on the Ramsey place, situated on the edge of the city of Grinnell. There were
the country, but clearly affected the grasses surrounding the bushes.
three marked zones about the bushes; in the first the grasses were badly infested with teleutospore stage; in the next there was less teleutospore stage and more of the uredospore; in the third there was only a trace of rust.

Fig. 19. A local epidemic developed about three bushes growing in an old orchard. The squirrel tail and red top near the bushes showed a very heavy infection of the uredospore stage on July 13, while the same grass beyond the shaded area was almost free from stem rust.

alba and *Agropyron repens* about the bushes was very severely infested with stem rust. The heavily rusted area extended 700 feet northwest from the bushes. About this area was another zone where the above grasses showed less of the teleutospore stage and more of the uredospore stage. The grain in the immediate vicinity was harvested before the final survey was made so no definite statements can be made as to the rust situation on the cereals. Be this as it may, the significant fact is, that a rust

epidemic started around the barberry hedge that was very marked and afforded ample opportunity for uredospore production which could readily be carried to surrounding grain fields.

An epidemic covering relatively a small area developed about three barberry bushes standing in an old orchard on a farm southeast of Des Moines. See fig. 19. Here the rust spread northwest from the bushes into a hollow in which there was a small run. On each side of this stream there was a good growth of grasses. The spread of the rust was limited on the north by the public highway. There was no grain within 40 rods of these barberry bushes.

DISCUSSION.

In the preceding pages eight cases have been described in which local stem rust epidemics developed about barberry plantings. In each case it was readily possible to find on the 1917 stubble or grasses teleutospores of *Puccinia graminis*. Sometime in the spring or early summer aecidia were found developing abundantly on the barberry bushes. In fact it was possible to find aecidia thruout July. About these bushes the grasses and grain became infested with stem rust earlier than similar grain and grasses some distance from barberry bushes. The host plants of stem rust in the immediate vicinity of the bushes were more heavily rusted and the teleutospores stage developed on them earlier than similar hosts further away. The cases at Riceville, Knoxville and Forest City serve to illustrate clearly how plantings in the outskirts of cities and towns may serve to start an epidemic. The evidence is quite complete showing that barberry bushes, either as hedges or isolated bushes, growing in the country or in the edge of cities may start local epidemics of stem rust. In the eight cases cited, the rust spread to all of our common cereals except rye. This exception may be due in part to the fact that barberry bushes growing near rye were not held under observation. However, in several of the cases described, *Agropyron repens* became heavily rusted, showing that the *P. graminis secalis* form was present.

It should be pointed out further that there probably occurred in the state more than the 16 local epidemics referred to in the preceding pages. It was quite impossible to make a careful survey about any considerable number of the plantings this past season. At the same time it should be said that doubtless many plantings failed to start infection centers due to a variety of causes such as the absence of viable teleutospores and susceptible host plants and unfavorable weather conditions. Be this as it may, the barberry served to start considerable number of local infection centers scattered over the state, that were limited in the area involved, only by the absence of favorable weather conditions. The eradication of the barberry would beyond all possibility of doubt stamp out these infection centers.

SUMMARY.

I. TELEUTOSPORE GERMINATION.

1. In the teleutospore germination studies in this bulletin *Puccinia graminis* on quack grass, wheat and volunteer oats germinated best when allowed to remain attached to the straw.

2. A relative humidity of at least 95.6 pct. is required for teleutospore germination and sporidia production. In 100 pct. relative humidity, however, sporidia production is more profuse. On the other hand teleutospores submerged in a drop of water produce long attenuated germ tubes and sporidia production is slight or wanting.

3. Sporidia production will take place at temperatures between 5°C. and 25°C. but most profusely at 20°C. At 5°C. the spores will push out a short germ tube and sporulate sparingly, while at the maximum temperature for germination, 30°C. the promycelium is abnormal in shape.

4. Germination takes place in about three hours after the spores have completed their rest period. Previous to and following this period the time is longer. The teleutospores have usually completed their rest period when the barberry leaf buds begin to open, altho the date at which they germinate freely, varies from one year to the other. This is in April or early May in this locality. After June 27, 1917 very little germination could be secured from spore material that had been held in the open.

5. Teleutospores germinate in the open following rains and the resulting promycelia can readily be seen as a white glistening growth on the surface of the sori. Teleutospores developed on volunteer grain in the late fall germinate more readily than those maturing in the summer or early fall. The viability of teleutospores can be prolonged to a certain extent by holding them at a low temperature.

6. The curvature of the promycelium is an adaptation resulting from differences of pressure within the basidial cells. The sterigmata are always so arranged on the curved promycelium as to direct the sporidia out away from the straw. They are projected with considerable force, being thrown up from the sorus 1000μ.

7. The sporidia germinate immediately after they are discharged from the promycelium. If their discharge is hindered they germinate while attached to the sterigmata and produce what has been called secondary sporidia. They are very susceptible to dry air and their germination seems to be most profuse between 15° and 20°C. Sporidia germinate readily on nutrient agars and have been kept alive and in a growing condition on them for four days but at the end of this period died.

II.—INFECTION.

1. Sporidia placed on barberry leaves under greenhouse conditions lead to infection and the production of mature aecidiospores in 12 days. When the barberry leaves have become dark green and crisp, infection apparently does not take place, due possibly to the increased thickness of the cuticle and walls of the epidermal cells. The germ tubes of the sporidia enter directly thru the cuticle and epidermis, altho the stomata are only on the under surface, both surfaces are susceptible to infection.

2. The germination of the teleutospores always seems to precede the advent of the aecidium on the barberry in the open. The aecidium has developed for the past three years from 12 to 45 days before the uredospore stage can be found on grains or grasses and in 1917 were most abundant in that part of the state where the rainfall was greatest. In 1918 aecidia were found generally thruout the state.

III. THE EUROPEAN BARBERRY.

In our survey, which is incomplete, to date 132,673 European barberry bushes were located on private and public grounds in 300 cities, also in towns and surrounding country in the state, over a third of these bushes have been eradicated. Only 10 out of the 29 largest nurseries are now carrying barberry nursery stock. The largest number of bushes were located in the larger cities as Dubuque, Davenport, Des Moines and Sioux City. More barberry bushes were found in the two tiers of counties along the Mississippi river than in all the rest of the state, not taking into consideration the nurseries.

IV. ESCAPED BARBERRY BUSHES.

The barberry escaped from cultivation in several localities in the state, notably at McGregor, Le Claire, Cascade, Garner and Montpelier. In no case, however, has the plant escaped far from the original planting, the farthest distance being about one mile. Birds seem to have been the chief agents of distribution. In every case where the shrub has escaped, it was found in native timber or planted groves. In only a few cases have bushes been found along fences. It seems to be unable to establish itself in open fields or pastures where trees do not occur.

V. DEVELOPMENT OF RUST.

The first appearance of the uredospore stage of stem rust during the last three years, was found to be as follows: 1916, May 21; 1917, June 15; and 1918, May 25. The teleutospores germinate freely before the first aecidia appear, and the uredospore stage on the grains and grasses develop after the aecidial stage on the barberry. In each case sufficient time intervenes

to permit the stage on the barberry to function and start the rust on grains.

VI. LOCAL EPIDEMICS.

1. During the summer of 1918, 16 local epidemics of stem rust infections were traced directly to the barberry. It attacked not only the various wild grasses but spread to oats, wheat and barley. In the case of oats and wheat serious damage resulted. The rust spreads in concentric zones in the direction of the prevailing wind. Infection centers varied from five to 320 rods in diameter. The eradication of the barberry would stamp out these infection centers.

2. Hedges in the country and bushes in the outskirts of towns were found to be the most serious offenders. Whether the barberry can aid the spread of stem rust seems to depend upon local conditions,—such as the close proximity of grasses, or straw well infested with the teleutospore stage, presence of susceptible grains and grasses, direction of prevailing wind, and favorable weather conditions for infection when aecidia are being produced on the barberry.

3. When near barberry bushes infected with aecidia, the following grasses are found infected with stem rust: *Hordeum jubatum*, *Agrostis alba*, and *Agropyron repens* and *A. tenerum*. These wild grasses, on account of their universal presence and long growing period, are a very important factor in the starting and aiding the spread of local epidemics of stem rust. They seem to serve as intermediate hosts between the barberry and cereals.

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